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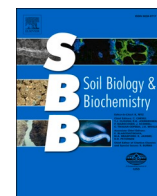


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Temperatures beyond the community optimum promote the dominance of heat-adapted, fast growing and stress resistant bacteria in alpine soils

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ABSTRACT

Alpine soils are warming strongly, leading to profound alterations in carbon cycling and greenhouse gas budgets, mediated via the soil microbiome. To explore microbial responses to global warming, we incubated eight alpine soils between 4 and 35 °C and linked the temperature dependency of bacterial growth with alterations in community structures and the identification of temperature sensitive taxa. The temperature optimum for bacterial growth was between 27 and 30 °C and was higher in soils from warmer environments. This temperature framing the upper limit of naturally occurring temperatures was a tipping point above which the temperature range for growth shifted towards higher temperatures together with pronounced changes in community structures and diversity based on both 16S rRNA gene and transcript sequencing. For instance, at the highest temperature, we observed a strong increase in OTUs affiliated with *Burkholderia-Paraburkholderia*, *Phenylobacterium*, *Pseudolabrys*, *Edaphobacter* and *Sphingomonas*. Dominance at high temperature was explained by *a priori* adaptation to high temperature, high growth potential as well as stress resistance. At the highest temperature, we moreover observed an overall increase in copiotrophic properties in the community along with high growth rates. Further, temperature effects on community structures depended on the long-term climatic legacy of the soils. These findings contribute to extrapolating from single to multiple sites across a large range of conditions.

1. Introduction

Alpine environments are particularly susceptible to climate change. In the European Alps, temperatures are projected to rise by 2–6 °C by 2085 compared to 1981–2010 with strongest warming in summer (Gobiet et al., 2014; CH2018, 2018). As 25% of the earth's surface is covered by mountains, biogeochemical processes in alpine environments considerably influence global greenhouse gas budgets (Donhauser and Frey, 2018). Microbes are resistant to harsh conditions prevailing in alpine environments, such as low and fluctuating temperatures, moisture fluctuations as well as high UV-radiation (Donhauser and Frey, 2018; Adamczyk et al., 2019). Thus, they are key players determining ecosystem functioning in these environments. However, how the ambient temperature governs microbial diversity and function in alpine ecosystems above the treeline remains largely unknown.

Bacterial temperature adaptation has been studied repeatedly using

incorporation of ³H-leucine as a proxy for bacterial growth, both in field and microcosm studies e.g. (Barcenas-Moreno et al., 2009; Rinnan et al., 2009; Rousk et al., 2012), although not in alpine ecosystems up to the nival zone. Three cardinal points define the temperature relationship of growth: The lower temperature limit for growth (T_{min}), the optimum where growth rates are highest (T_{opt}) and the upper thermal limit for growth (T_{max}). Typically, growth rates increase quadratically up to T_{opt} and sharply decline at higher temperatures in pure cultures (Ratkowsky et al., 1983) and environmental communities (Rinnan et al., 2009; Rousk et al., 2012; Birgander et al., 2013). T_{min} was found to correlate with the mean annual temperature of the environment (Rinnan et al., 2009; Nottingham et al., 2019) and is thought to be between –15 and –10 °C for polar environments, between –5 and –10 °C for temperate environments and between –5 and 0 °C for tropical environments (Bååth, 2018). Below 0 °C, the reduced availability of liquid water affects growth. As T_{min} is inferred from extrapolation of measurements

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above 0 °C, it is a theoretical concept, but nonetheless a useful descriptor of temperature adaptation. T_{opt} has been shown to be substantially higher than normal *in-situ* temperatures (Barcenas-Moreno et al., 2009; Rinnan et al., 2009; van Gestel et al., 2013; Birgander et al., 2018). It has been hypothesized that fast proliferation of heat-adapted taxa and strong impairment of heat-sensitive taxa rapidly lead to dominance of the former at high temperature. Conversely, low temperatures do not exert sufficient selective pressure to lead to takeover of cold-adapted taxa throughout the course of the year (Rousk and Bååth, 2011; van Gestel et al., 2013). However, the changes in microbial community structures underlying changes in temperature adaptation of growth rates have not been investigated to date. To address this gap, we combine measurement of bacterial growth rates with an in depth assessment of diversity and community structures.

Changes in the microbial community structure can lead not only to a shift in community level temperature adaptation but also in growth strategy: copiotrophs are able to respond quickly to favorable environmental conditions and grow rapidly upon availability of labile C sources. Conversely, oligotrophs exhibit slow, continuous growth under low C and nutrient regimes with a high yield of biomass per unit substrate (Roller and Schmidt, 2015; Ho et al., 2017). As these growth strategies are potentially associated with the amount of CO₂ emissions, in this study, we address whether shifts between oligotrophy and copiotrophy occur in response to temperature.

Previous studies mostly addressed warming responses at a crude taxonomic resolution, yielding inconsistent results across studies (de Scally et al., 2016; Mateos-Rivera et al., 2016). However, temperature adaptation is conserved in the bacterial phylogeny only at a shallow level (Martiny et al., 2015). In addition, the temperature sensitivity of microbial community structures and the threshold at which direct temperature effects become apparent has rarely been linked with the ambient temperature regime. As alpine ecosystems harbor sharp climatic gradients within short spatial distance, they provide ideal conditions to study temperature adaptation of soil microbial communities keeping other environmental parameters such as bedrock comparable (Pauli, 2015; Adamczyk et al., 2019).

Here, in a laboratory experiment, we compare the temperature response of bacterial communities in eight alpine soils with different microclimatic legacies associated with altitude and aspect, resulting in a gradient of mean annual temperatures spanning about 7 °C. We link temperature adaptation at the functional level (³H-leucine incorporation) with amplicon sequencing data of 16S rRNA genes and transcripts at high taxonomic resolution. To create a framework for predicting temperature responses, we incubated these soils for one month at five different temperatures between 4 and 35 °C, covering the current range of ambient temperatures plus 10 °C representing an increase in

maximum temperature with global warming.

2. Materials and methods

2.1. Sites description and sampling

We sampled soil from four alpine summits at the north and south aspect, respectively. The sites are located in the eastern Swiss Alps (coordinates and properties of the sites are summarized in Table 1) and situated above the treeline ranging from the alpine zone to the largely plant free nival zone. Our sites are partly based on the Swiss National Park 2 (SN2) sites from the GLORIA (GLOBal Observation Research Initiative in Alpine environments) project (Pauli, 2015). The bedrock is siliceous leading to the development of relatively acid soils (pH 3.87–4.59, Table 1). The treeline in the area is at approx. 2300 m a.s.l. At site 1, some dwarf shrubs grow, while site 2–4 are dominated by mosses and lichens on the north slope and by grasses on the south slope. Overall, at site 3 and 4, especially on the north slopes, vegetation is very sparse (<50% cover). We have monitored soil temperatures at 5 cm depth since August 2016. The sites are characterized by a mean annual soil temperature (MAST) between −1.9 and 4.8 °C with a minimum of −17.3 °C in the coldest soil and a maximum of 27.2 °C in the warmest soil (Table 1). The mean growing season temperature (June–September) ranges from 3.7 to 11.0 °C. Carbon and nitrogen content range from 0.43 to 3.48 and 0.03–0.28%, respectively. Five replicate soil samples were taken within a radius of 5 m from approximately 2–20 cm depth removing plants and roots as thoroughly as possible. Samples were transported to the lab as cool as possible, sieved at 4 mm mesh size and subsequently stored at 4 °C. Sampling took place in August 2016, the experiment for this study was conducted in March 2017.

2.2. Conduction of the temperature incubation experiment

The experimental set-up is shown in Fig. S1. The experiment was conducted in triplicates, which were prepared from the five field replicates pooled at equal weight and split into three subsamples per condition: the water content of each soil was adjusted to 60% of the water holding capacity with sterilized water and 20 g dry soil equivalent were distributed to autoclaved 100 ml Erlenmeyer flasks. All preparations took place at 4 °C. Moreover, an aliquot was frozen as a time 0 control for DNA and RNA analysis (samples for RNA were shock frozen in liquid nitrogen). The Erlenmeyer flasks were sealed with sterilized cellulose plugs allowing for aeration while preventing contamination. We incubated the soils for 28 days at 4, 9, 15, 25 and 35 °C, respectively. The temperature was elevated by 1.5 °C every 30 min until the incubation temperature was reached and then kept stable. 4 °C represents the

Table 1
Site characteristics.

	Coordinates	Altitude [m a.s.l.]	MAST [°C]	MGSSST [°C]	T _{min} [°C] (soil)	T _{max} [°C] (soil)	% TC	% TN	pH	% Sand	% Silt	% Clay	Water holding capacity [L/g ¹ dry soil]
Site 1 N	N46° 43.004' E10° 21.264'	2464	1.30	7.88	−10.08	13.96	3.30	0.19	3.87	84	13	3	0.75
Site 1 S	N46° 42.959' E10° 21.279'	2465	4.77	10.98	−6.01	27.24	2.58	0.19	3.75	74	19	6	0.65
Site 2 N	N46° 41.639' E10° 19.770'	2777	−1.71	5.07	−17.31	19.43	1.00	0.08	3.97	85	12	3	0.45
Site 2 S	N46° 41.632' E10° 19.775'	2777	4.08	9.70	−3.46	24.40	3.48	0.28	3.96	82	13	4	0.80
Site 3 N	N46° 29.780' E9° 55.887'	2960	−1.92	3.65	−10.50	12.81	0.34	0.03	4.59	90	7	4	0.32
Site 3 S	N46° 29.753' E9° 55.936'	2960	1.12	6.39	−11.83	20.48	0.82	0.05	4.52	87	11	3	0.33
Site 4 N	N46° 42.525' E10° 23.307'	3092	−1.70	5.71	−16.17	19.19	0.43	0.04	4.15	90	8	3	0.31
Site 4 S	N46° 42.456' E10° 23.322'	3049	0.93	7.39	−15.33	22.33	1.63	0.13	3.98	84	12	4	0.46

control at storage temperature. Microbial communities at 4 °C were largely identical to the time 0 control, thus, the time 0 is not shown in the results section. Water loss was compensated gravimetrically with sterilized water daily for 25 and 35 °C treatments, every two days for the 9 and 15 °C treatments and weekly for the 4 °C treatment. The maximum water loss due to evaporation amounted to about 10% of the total water. After the incubation, soil samples were harvested as quickly as possible to minimize temperature effects during handling of the samples. Aliquots for RNA extraction were frozen immediately in liquid nitrogen, aliquots for DNA extraction were frozen at -20 °C, and soil samples for the measurement of ³H-leucine incorporation were immediately stored at 4 °C and cooled during transport to the university of Zurich where the measurements were conducted within 1 h after harvest.

2.3. Temperature dependent measurement of ³H-leucine incorporation

In order to investigate bacterial temperature adaptation, we assessed the temperature dependence of ³H-leucine incorporation as a proxy for bacterial growth. These measurements were conducted according to the method adapted for soil described by Bååth et al. (2001). Briefly, a bacterial suspension in water was produced from 5 g of soil in 20 ml sterilized, deionized water by vortexing for 30 s at maximum speed followed by 10 min centrifugation at 1000g. ³H-leucine (37MBq ml⁻¹ and 5.74 TBq mmol⁻¹; Perkin Elmer, USA) mixed with unlabelled leucine resulting in a final concentration of 275 nM was then added to 1.5 ml bacterial suspension. To measure the temperature dependence of leucine incorporation for each soil from the experiment, from the 20 ml soil suspension obtained from each soil, we prepared 7–8 aliquots of 1.5 ml. After adding the radiolabeled tracer, the aliquots from soil samples after the incubation treatment (time 1) were incubated at 4, 12, 18, 25, 30, 35 and 40 °C for incubation temperatures up to 25 °C and in addition at 45 °C for the 35 °C incubations, respectively. Hereafter, we refer to these temperatures as measurement temperatures. At time 0 (before the incubation treatment), due to logistic constraints 7 °C was used instead of 4 °C and 40 °C was excluded. Incubation times at different temperatures were chosen to obtain a similar signal across all samples while minimizing alterations in temperature adaptation during the measurements (Rinnan et al., 2009). Thus, the 4 °C (or 7 °C) samples were incubated for 18 h, the 12 °C samples for 6 h and all other sample for 2 h ³H-leucine incorporation was terminated by adding trichloroacetic acid. After removal of excess leucine and several washing steps, the ³H-activity of incorporated leucine was determined by liquid scintillation counting. Subsequently, we calculated ³H-activity, which is proportional to leucine incorporation, per h and g dry soil.

2.4. Calculation of temperature dependent properties of ³H-leucine incorporation (T_{min} , T_{opt} , Q_{10})

The temperature relationship of bacterial growth was assessed using the Ratkowsky square root model according to equation (1) (Ratkowsky et al., 1982, 1983) which has been shown to adequately model bacterial growth both in cultures (Ratkowsky et al., 1983; Ross et al., 2011) and environmental samples (Rinnan et al., 2009; Birgander et al., 2013, 2018).

$$\sqrt{Leu} = a(T - T_{min}) \times \left(1 - e^{b(T - T_{max})}\right) \quad (1)$$

where Leu is the ³H-leucine incorporation rate, T is the temperature, a and b are slope parameters, T_{min} is the minimum temperature for growth and T_{max} is the maximum temperature for growth. Below the optimum temperature the equation can be simplified to a linear function:

$$\sqrt{Leu} = a(T - T_{min}) \quad (2)$$

According to Ratkowsky et al. (1983), we used equation (2) to estimate a and T_{min} (T_{min} is the intercept with the x-axis), which were then

used as constants to fit the model over the entire temperature range (equation (1)). Q_{10} (the ratio of a process at two temperatures at an interval of 10 °C) describes the temperature sensitivity and was calculated according to equation (3):

$$Q_{10} = \frac{Leu(T + 10)}{Leu(T)} = \left(\frac{T + 10 - T_{min}}{T - T_{min}}\right)^2 \quad (3)$$

Leu(T) is the rate of ³H-leucine incorporation at a given temperature and Leu(T+10) is the rate of ³H-leucine incorporation at 10 °C higher. We calculated Q_{10} over temperature intervals relevant for the field conditions in our soils.

2.5. Extraction of nucleic acids, reverse transcription, PCR and sequencing

Total DNA was extracted with the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA was extracted using the RNeasy PowerSoil Total RNA Kit (Qiagen, Hilden, Germany) from 2 to 5 g soil according to the manufacturer's instructions. RNA samples were cleaned with the RNeasy PowerClean Pro CleanUp Kit (Qiagen, Hilden, Germany) and subsequently remaining DNA contamination was removed using the DNase Max Kit (Qiagen, Hilden, Germany). DNA was quantified with PicoGreen (Invitrogen, Carlsbad, CA, United States), RNA was quantified using the RNA specific Qubit RNA HS Assay Kit RNA (Invitrogen, Carlsbad, CA, United States). Complete removal of genomic DNA was ensured by PCR amplification of the bacterial 16S rRNA gene from the RNA samples. Subsequently, RNA was transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with the maximum possible amount of template RNA or the maximum volume for samples with low concentrations. The V3 – V4 region of the bacterial 16S rRNA gene was PCR amplified in triplicate reactions using the primer pair 341F and 806R and PCR conditions as described previously (Frey et al., 2016). 10 ng of template DNA or cDNA was used per PCR reaction. Triplicate PCR reactions were pooled and sent to the Génome Québec Innovation Center at McGill University (Montréal, Canada) for barcoding using the Fluidigm Access Array technology (Fluidigm) and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA).

2.6. Quantitative real time PCR

Copy numbers of the 16S rRNA gene were measured by quantitative real-time PCR (qPCR) using 2 ng of template DNA with primers and PCR conditions as described previously (Rime et al., 2015). Standard curves were obtained in triplicates.

2.7. Sequence quality control, OTU clustering, taxonomic assignments and prediction of 16S rRNA gene copy numbers

Quality filtering, OTU clustering and assignment was conducted similarly as described previously (Frey et al., 2016) using a customized pipeline based on UPARSE (Edgar, 2013). Briefly, paired-end reads were merged using the fastq_mergepairs algorithm (Edgar and Flyvbjerg, 2015), filtering for sequences with a minimum length of 300 bp and a minimum overlap of 50 bp. PCR primers were removed using Cutadapt (Martin, 2011) allowing for a maximum of one mismatch in the forward and reverse primer. Next, reads were quality filtered using the USEARCH fastq_filter function discarding reads with an expected error of one or greater. Dereplicated sequences were clustered into OTUs at 97% sequence identity using the cluster_otu function. The clustering step includes an "on the fly" chimera removal algorithm. The OTU centroid sequences were then filtered for the presence of ribosomal signatures using Metaxa2 (Bengtsson-Palme et al., 2015). Subsequently, sequences were mapped back on the OTU centroid sequences and

taxonomic classification was conducted using a naïve Bayesian classifier (Wang et al., 2007) implemented in Mothur (Schloss et al., 2009) with a minimum bootstrap support of 0.6, querying against SILVA v128 (Quast et al., 2013). Raw sequences were deposited in the NCBI Sequence Read Archive under the accession number PRJNA611981.

16S rRNA gene copy numbers per genome as a marker for bacterial trophic strategy (Klappenbach et al., 2000) were predicted using PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states (Langille et al., 2013)). PICRUSt is a tool used to predict metagenomes based on an ancestral-state reconstruction algorithm using marker gene data and a reference database containing fully sequenced bacterial genomes.

2.8. Statistical analyses and visualization of results

All statistical analyses were conducted using R (R Core Team, 2018) and primerE (Clarke and Gorley, 2006). R code used for this manuscript is available in the supplementary material. All plots were created using ggplot (Wickham, 2009).

We linearly regressed properties related to leucine incorporation before the incubation treatment (time 0) against MAST to determine the effect of the climatic legacy. We also regressed against the mean growing season temperature, which we assumed to be more relevant in shaping microbial communities and biological activity. However, results were largely the same as for MAST. Therefore, for reasons of comparability with previous studies, we chose to represent only regressions against MAST.

To assess the effect of the incubation treatment (time 1), we used a one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (Tukey HSD) post-hoc tests to compare different temperatures in subsets for each soil. We used the Shapiro-test and the Bartlett-test to test for normality and homoscedasticity, respectively, and log-transformed to fulfill the assumptions of ANOVA if necessary.

For the analysis of bacterial communities, alpha-diversity indices were calculated based on OTU abundance matrices rarefied to even sequencing depth. We performed a one-way ANOVA to test for significant differences in observed richness and Shannon diversity in response to incubation temperature for each soil separately, followed by Tukey HSD test. Similarly, differences in 16S rRNA copy numbers (qPCR data) as well as average estimated 16S rRNA gene copy numbers per genome per sample (PICRUSt data) were estimated by ANOVA and Tukey HSD test. We tested if the assumptions of ANOVA were satisfied as described above and again log-transformed the data if necessary. Principal coordinate analyses (PCoAs) were calculated based on Bray-Curtis distances using square-root transformed relative abundances. Differences in community structures with temperatures were assessed by permutational multivariate analysis of variance (PERMANOVA) in the subset for each soil. Pairwise comparisons between each temperature and the 4 °C control were conducted using Monte-Carlo simulations in primerE (Clarke and Gorley, 2006). Finally, we used Mantel test to compare bacterial community structures at the DNA and at the RNA level. To assess if changes in observed richness were related with the natural climatic legacy of the soils, we calculated the ratio between richness at the respective treatment temperature and the 4 °C control, which we linearly regressed against MAST. To determine the relationship between the impact of the incubation temperature on bacterial community structures and the climatic legacy at the sites, linear regression of the mean Bray-Curtis distance between the respective incubation temperature and 4 °C against MAST was used.

Co-occurrence and mutual exclusion patterns were calculated at each temperature across our eight soils with three replicates (in total 24 samples per temperature) using Spearman rank correlations of relative abundances. We used Benjamini-Hochberg corrections for multiple comparisons. For the subsequent network analysis, at each temperature, we included OTUs with at least one correlation with a $p_{adj} < 0.01$, a correlation coefficient < -0.7 or > 0.7 , and occurring in more than 10

out of 24 samples. The R-package igraph (Csardi and Nepusz, 2005) was used for constructing networks at each temperature. The OTUs are the nodes of the network and they are connected by an edge if their abundance is correlated (fulfilling the criteria above). Networks were calculated separately for co-occurrence patterns based on positive correlations and mutual exclusion patterns based on negative correlations. The node degree represents the number of edges connecting it to other nodes. Network density represents the ratio of edges present in the network to the maximum number of edges possible and reflects how connected the network is.

In order to assess which OTUs were significantly affected by the incubation temperature, for each treatment temperature, we calculated \log_2 fold changes relatively to the 4 °C control at the DNA level, using DESeq2 (Love et al., 2014). As we used the RNA data to confirm viability of the OTUs identified as significantly changing in abundance in response to the treatment temperature, for this analysis, we used the four soils where both DNA and RNA was analyzed (site 2 N and S, site 4 N and S). We controlled for the effect of site and aspect and calculated \log_2 -fold changes between incubation temperatures.

3. Results

3.1. Bacterial growth

3.1.1. Relationship with the natural temperature regime (time 0)

For our experiment, we used soils with different climatic legacy (from four summits at different altitudes, north and south exposed, respectively) resulting in a gradient of mean annual soil temperature (MAST) from -1.9 °C to 4.8 °C. First, we characterized the temperature adaptation of bacterial growth before the incubation treatment using incorporation of ^3H -leucine, in order to relate descriptors of temperature adaptation with the natural climatic legacy. Below the optimum (temperatures up to 25 °C), the relationship between square root transformed leucine incorporation and measurement temperature was well represented by a linear regression. We obtained R^2 values > 0.97 in 75% of the samples and > 0.9 in all samples. T_{\min} ranged from -11.6 to -4.2 °C (Fig. 1A, Table 2) and increased by 0.71 per °C increase in MAST ($R^2 = 0.43$, $P = 0.08$, Table 3). T_{opt} where maximum leucine incorporation occurred was between 27.3 and 30.3 °C (Fig. 1A, Table 2) and also increased with MAST ($R^2 = 0.28$, $P = 0.17$, increase per °C = 0.18). The Q_{10} ranged from 2.1 to 2.9 between 10 and 20 °C and from 3.48 to 11.35 between 0 and 10 °C (Table 2). Both correlated positively with MAST (Q_{10-20} : $R^2 = 0.35$, $P = 0.12$; Q_{0-10} : $R^2 = 0.2$, $P = 0.27$). Q_{10-20} increased by 0.063 per °C increase in MAST and Q_{0-10} by 0.47 (Table 3).

3.1.2. Response to the incubation temperature (time 1)

To assess the response to the incubation temperature, we measured leucine incorporation after the one month incubation. These measurements were done in four of the eight soils (site 2 N and S and site 4 N and S). Also here, the temperature dependence was well modelled by a linear relationship with square root transformed leucine incorporation. The R^2 was > 0.97 in 78% of the samples and > 0.9 in all samples except one. In all four soils, T_{opt} remained constant up to 15 °C but increased at 25 and 35 °C (Table 4; Fig. 1B shows site 2 N as an example, Fig. S2 shows all four soils). T_{\min} and Q_{10-20} changed only after incubation at 35 °C where also overall leucine incorporation was substantially higher than at lower temperatures (Table 4, Fig. S3).

3.2. Temperature effects on bacterial abundance, diversity, community structures and network properties

3.2.1. Abundance and diversity

To relate changes in growth rates and bacterial community structures with bacterial abundance we determined 16S rRNA gene copy numbers. They did not show a significant shift across incubation temperatures in any of the soils (Fig. S4). Next, we investigated bacterial

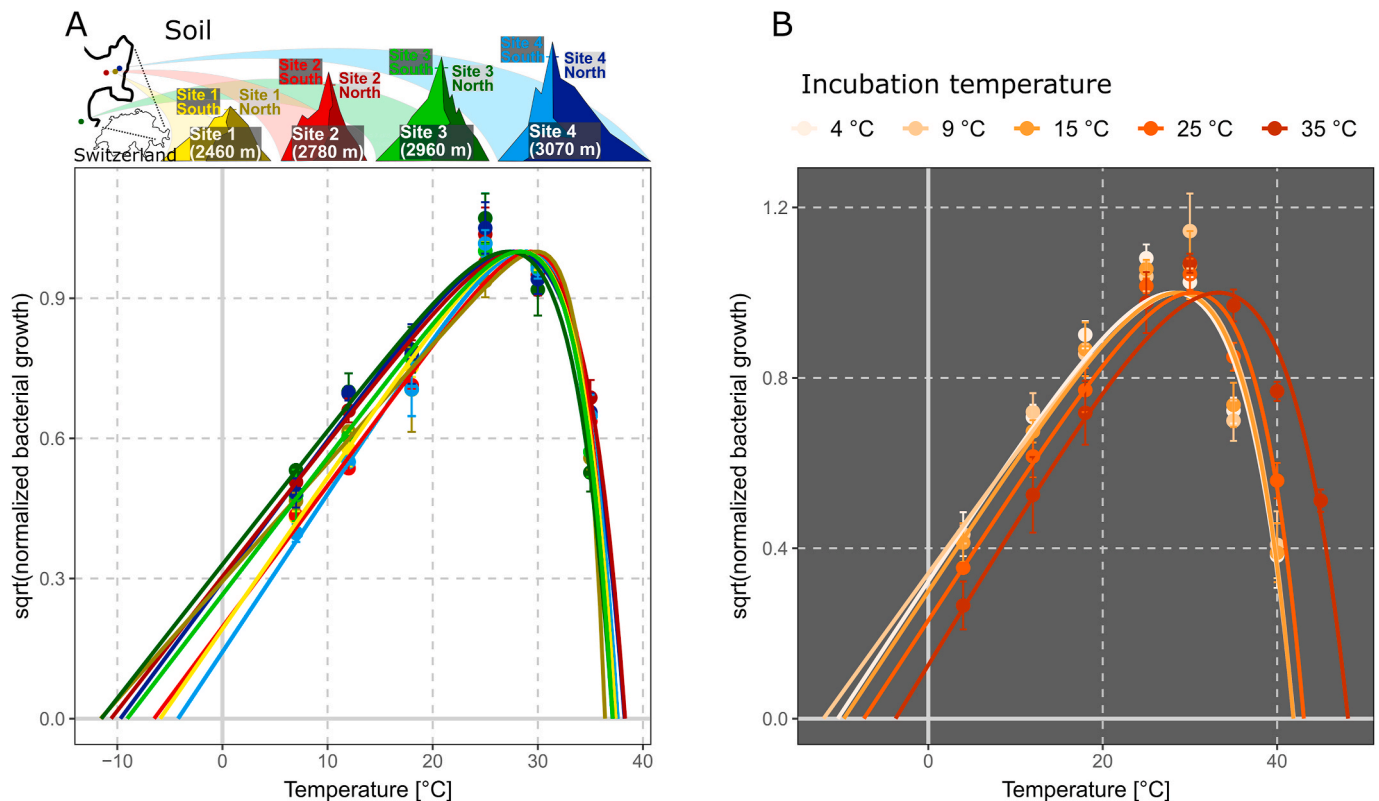


Fig. 1. Temperature dependency of bacterial growth rates. Square root transformed bacterial growth rates (assessed by ^3H -leucine incorporation) normalized to the growth rate at T_{opt} plotted against measurement temperature. (A) The different soils before the experiment (time 0), (B) Site 2 N after exposure to different incubation temperatures (time 1); all four soils after the incubation are shown in Figs. S2 and S3. Values represent the mean \pm SD ($n = 2$ for soils before incubation, $n = 3$ for soils after the incubation); DPM = disintegrations per minute.

Table 2

Characteristics of the temperature adaptation of bacterial growth analyzed using ^3H -leucine incorporation before the incubation (time 0).

	T_{min}	T_{opt}	Q_{10-20}	Q_{0-10}
Site 1 N	-11.63 ± 1.13	30.24 ± 1.58	2.14 ± 0.07	3.48 ± 0.31
Site 1 S	-5.92 ± 0.27	28.45 ± 0.07	2.65 ± 0.03	7.24 ± 0.41
Site 2 N	-10.69 ± 2.62	28.34 ± 0.95	2.21 ± 0.18	3.89 ± 0.93
Site 2 S	-6.50 ± 0.04	29.38 ± 0.13	2.58 ± 0.00	6.45 ± 0.05
Site 3 N	-11.54 ± 0.95	27.34 ± 0.96	2.15 ± 0.06	3.50 ± 0.27
Site 3 S	-9.24 ± 3.32	28.53 ± 0.55	2.34 ± 0.28	4.74 ± 1.79
Site 4 N	-10.02 ± 3.69	27.91 ± 0.32	2.28 ± 0.28	4.37 ± 1.63
Site 4 S	-4.22 ± 0.01	28.86 ± 0.48	2.90 ± 0.00	11.35 ± 0.03

Values represent the mean \pm SD ($n = 2$). T_{min} = minimum temperature for bacterial growth, T_{opt} = optimum temperature for bacterial growth, $Q_{10-20} = Q_{10}$ between 10 °C and 20 °C, $Q_{0-10} = Q_{10}$ between 0 and 10 °C, DPM = disintegrations per minute. Altitude of the sites: Site 1: 2460 m a.s.l., Site 2: 2780 m a.s.l., Site 3: 2960 m a.s.l., Site 4: 3070 m a.s.l. N = North, S = South.

diversity. We obtained 7 828 212 high quality sequences ($36\,242 \pm 9058$ per sample) that clustered into 12 921 OTUs (1953 ± 609 per sample). At 4 °C, we found between 956 and 1708 OTUs in the different soils. This number remained constant up to 15 °C, decreased slightly at 25 °C and strongly at 35 °C (Fig. 2A). The same pattern was found for Shannon diversity (Fig. S5). In the four soils where we analyzed RNA, approx. 60% of the OTUs found at the DNA level were also found at the RNA level at all incubation temperatures (Fig. S6).

To test the hypothesis that soils with a lower *in-situ* temperature react more strongly to the incubation temperature, we regressed the percentage of OTUs remaining at 25 °C and 35 °C incubation temperature (where significant changes occurred) compared to 4 °C against MAST. Here, we found a significant correlation at 35 °C ($R^2 = 0.921$, $P = 0.001$) but not at 25 °C ($R^2 = 0.442$, $P = 0.27$, Table 3).

Table 3

Relationship between descriptors of bacterial temperature adaptation (T_{min} , Q_{10-20} , Q_{0-10} , T_{opt}) as well as treatment effect on diversity and community structure and the natural temperature regime of the soils.

Leucine incorporation			
	R^2	P	Increase per °C
T_{min} - MAST	0.43	0.079	0.709
Q_{10-20} - MAST	0.35	0.121	0.063
Q_{0-10} - MAST	0.20	0.266	0.465
T_{opt} - MAST	0.28	0.174	0.183
Alpha-diversity (% richness at 25 and 35 relative to 4 °C)			
	R^2	P	
% richness 25 °C/4 °C - MAST	0.20	0.273	
% richness 35 °C/4 °C - MAST	0.85	0.001**	
Beta-diversity (mean distance to 4 °C)			
	R^2	P	
distance 15 °C/4 °C - MAST	0.17	0.312	
distance 25 °C/4 °C - MAST	0.59	0.026*	
distance 35 °C/4 °C - MAST	0.76	0.005**	

R^2 and P values are derived from linear regression between the mean of replicates and the mean annual soil temperature (MAST). T_{min} = minimum temperature for bacterial growth, T_{opt} = optimum temperature for bacterial growth, $Q_{10-20} = Q_{10}$ between 20 °C and 10 °C, $Q_{0-10} = Q_{10}$ between 0 and 10 °C, MAST = mean annual soil temperature. n.s. not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3.2.2. Community structure

In all soils, the community structures shifted gradually with incubation temperature, with the most pronounced shift at high temperatures (Fig. 2B). Both at the DNA and RNA level, community structures at

Table 4Characteristics of the temperature adaptation of bacterial growth analyzed using ^3H -leucine incorporation after the incubation (time 1).

	T_{\min}		Q_{10-20}		T_{opt}		
	4 °C	35 °C	4 °C	35 °C	4 °C	25 °C	35 °C
Site 2 N	-10.45 ± 1.86	-3.67 ± 1.71**	2.23 ± 0.14	3.03 ± 0.34**	28.18 ± 0.53	30.41 ± 0.37*	33.35 ± 1.10***
Site 2 S	-5.78 ± 2.09	-1.63 ± 1.26n.s.	2.70 ± 0.26	3.49 ± 0.37*	28.42 ± 0.74	29.99 ± 0.54	33.92 ± 1.04***
Site 4 N	-7.94 ± 2.09	-0.78 ± 0.48*	2.44 ± 0.19	3.72 ± 0.15***	28.13 ± 0.36	31.25 ± 0.65**	35.23 ± 1.21***
Site 4 S	-5.04 ± 2.75	-4.99 ± 1.31n.s.	2.83 ± 0.37	2.79 ± 0.20n.s.	29.64 ± 0.33	33.38 ± 1.32***	34.06 ± 0.46***

Temperatures are incubation temperatures (temperatures where significant changes compared to 4 °C occurred are shown). Asterisks indicate significant differences at the respective treatment temperature compared to 4 °C (Significance levels: n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Values represent the mean ± SD ($n = 3$). T_{\min} = minimum temperature for bacterial growth, T_{opt} = optimum temperature for bacterial growth, $Q_{10-20} = Q_{10}$ between 10 °C and 20 °C, DPM = disintegrations per minute. Altitude of the sites: Site 1: 2460 m a.s.l., Site 2: 2780 m a.s.l., Site 3: 2960 m a.s.l., Site 4: 3070 m a.s.l. N = North, S = South.

35 °C were different from those at 4 °C in all sites and at 25 °C in most of the sites but not at 15 and 9 °C except for site 3 N at 15 °C (Table 5). In the subsets per soil, Mantel test revealed highly similar community structures over the temperature gradient at the DNA and RNA level (Table 5).

To test whether the community structures soils with a lower *in-situ* temperature react more strongly to the incubation temperature, we regressed the mean Bray-Curtis distance between each incubation temperature and 4 °C against MAST, yielding significant correlations for 25 and 35 °C incubation temperature ($R^2 = 0.59$, $P = 0.026$ and $R^2 = 0.76$, $P = 0.005$, Table 3).

3.2.3. Co-occurrence and mutual exclusion network properties

To explore the relationship among different bacterial taxa in response to the incubation temperature, we investigated co-occurrence (based on positive correlations) and mutual exclusion patterns (based on negative correlations) among OTUs across the temperature gradient using network analysis. OTUs represent the nodes of a network and two OTUs are connected with an edge if their abundance is significantly correlated. The node degree is the number of edges connected to a certain node. For all temperatures, it followed a power-law distribution for both co-occurrence and mutual exclusion networks, which indicates that the networks are scale-free (Fig. 3). However, for the co-occurrence network the distribution did not show a completely smooth decrease with increasing degree, but displayed a small peak for degrees of approximately 170. While the degree showed no pattern among incubation temperatures for the co-occurrence networks, it strongly decreased with increasing temperature for the mutual exclusion networks. Network density, indicating how strongly the network is connected, was higher at 35 °C compared to lower temperatures for the co-occurrence network, whereas it tended to decrease with increasing incubation temperature at high temperatures for the mutual exclusion networks (Fig. 3).

3.3. Temperature effects on bacterial growth strategies

To identify changes in trophic strategy (copiotrophy versus oligotrophy) in the bacterial community, we calculated the average 16S rRNA gene copy number per genome in each sample. In five of our eight soils the 16S rRNA gene copy number per genome was significantly higher at 35 °C compared to lower incubation temperatures indicating an increase in copiotrophic bacteria (Fig. 4).

3.4. Identification of temperature sensitive taxa

In order to identify temperature sensitive taxa, we estimated \log_2 -fold changes (LFC) with incubation temperature across different soils (in the subset of soils where both DNA and RNA were analyzed) controlling for the effect of site and aspect and contrasting the respective temperature with 4 °C. At 15 °C, we found 22 differentially abundant OTUs of which 12 increased and 10 decreased and whereof 10 could be classified at the genus level (Table S1). At 25 °C, 239 OTUs displayed a significant

LFC (36 increasing and 203 decreasing, 106 classified at the genus level). At 35 °C, we observed 1067 differentially abundant OTUs (162 increasing and 905 decreasing, 408 classified at the genus level). While at 15 and 25 °C, almost all OTUs were found at the RNA level, at 35 °C all of the increased OTUs were present at the RNA level but many of the decreased OTUs were not. The abundance of OTUs belonging to Bacteroidetes, especially within the genera *Mucilaginibacter* and *Cytophaga* was reduced consistently at all three treatment temperatures with increasingly strong LFC with increasing temperature (Fig. 5A). Similarly, most genera within Actinobacteria decreased at 35 °C, with the exception of two OTUs affiliated with the genus *Acidothermus*. Of the genera reduced at 35 °C within Actinobacteria only the abundance of *Lysinimonas* was slightly reduced at 25 °C. In addition, several members of Firmicutes such as *Bacillus* and *Clostridium* slightly increased in abundance at 35 °C compared to 4 °C. Deltaproteobacteria consistently showed a moderate decrease at 25 and a stronger decrease at 35 °C (Fig. 5A). The same pattern was observed for Planctomycetes except for two OTUs within *Singulisphaera* at 25 °C and one OTU within *Gemmata* at 35 °C.

The OTUs that increased most strongly at 35 °C consistently displayed a very low abundance up to 15 °C, increased slightly at 25 °C in a few cases and then increased strongly at 35 °C representing up to 20% of the sequences at this temperature corresponding to an LFC up to 10 compared to 4 °C (Fig. 5, Fig. S7A). These OTUs belonged to *Burkholderia-Paraburkholderia* as well as *Phenyllobacterium*, *Pseudolabrys*, *Edaphobacter* and *Frateriia*. OTUs within *Burkholderia-Paraburkholderia* and *Phenyllobacterium* increased moderately at 25 °C (up to an LFC of 2) while the abundance of *Pseudolabrys*, *Edaphobacter* and *Frateriia* was unchanged at temperatures below 35 °C. Compared to the enriched OTUs, the most strongly decreasing OTUs at 35 °C showed a more gradual decrease over the temperature gradient with most OTUs showing a clearly decreased abundance already at 25 °C (Figs. 5B and S7B).

At the RNA level, we largely observed identical patterns (Fig. S8). The main difference was that among Firmicutes only *Acidibacillus* increased at 35 °C and that among Deltaproteobacteria the genera *Haliangium* and *H16* comprised OTUs that increased at 35 °C. Similarly, when all eight soils were included the main pattern was identical confirming the consistency of warming responses across different soils (Fig. S9). Only within Bacteroidetes, in contrast to the subset, we also found OTUs that increased at 35 °C and that belonged to *Flavitalea*, *Mucilaginibacter*, *Candidatus amoebophilus* and *Segetibacter*. In order to assess consistency of the changes observed in the combined analysis, we also calculated \log_2 -fold changes individually for the soils shown in Fig. 5A (site 2 N and S, site 4 N and S) and compared them with the combined results in Fig. 5A (Fig. S10). Overall, the direction of change was consistent across sites, however the magnitude of the LFC varied. For instance, in site 2, the LFC of an OTU affiliated with *Burkholderia-Paraburkholderia* at 35 °C was 14, while it was less than 10 for the other soils. Also among the replicates, changes in abundances varied considerably for some OTUs (Fig. S7). Disagreeing direction of change was mostly found only for OTUs that did not have a significant LFC in all of

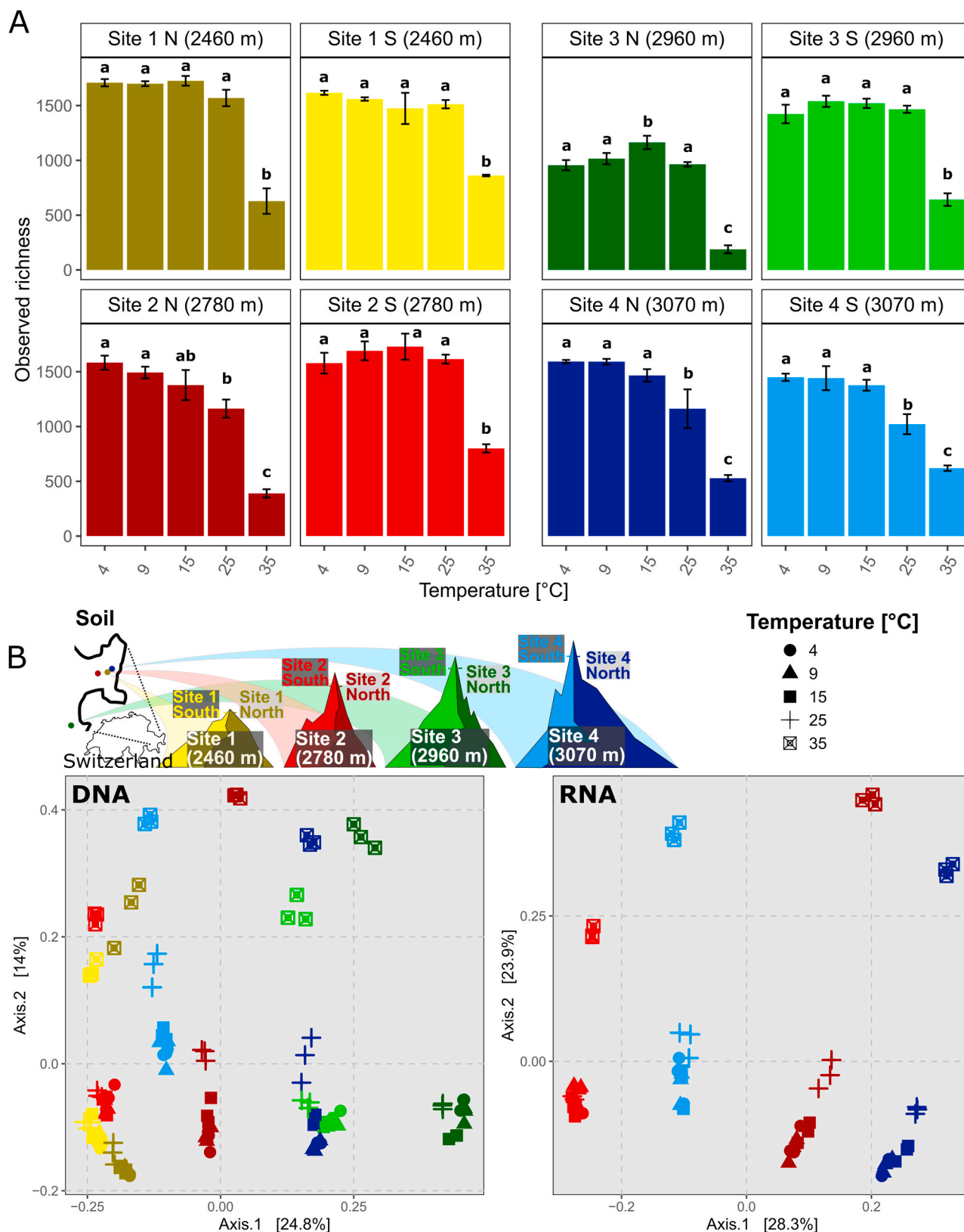


Fig. 2. Temperature effects on diversity and community structures A) Observed richness (mean \pm SD, $n = 3$) of bacterial OTUs at the DNA level in each soil at the different incubation temperatures. Different letters indicate a significant ($P < 0.05$) difference between temperatures as assessed by one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test in the subsets per soil. B) Principal coordinate analysis (PCoA) of bacterial community structures at the DNA level (left) and RNA level (right). Ordinations were based on Bray–Curtis dissimilarities square root-transformed relative abundances. The variation explained by each PCoA axis is given in parentheses.

Table 5

Effect of incubation temperature on bacterial community structures based on 16S rRNA genes (DNA) and 16S rRNA transcripts (RNA).

DNA (PERMANOVA)								
	Site 1 N		Site 1 S		Site 2 N		Site 2 S	
Overall ($F_{4,14}$, P)	7.47	<0.0001***	4.13	<0.0001***	11.23	<0.0001***	4.06	<0.0001***
Pairwise	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)
4 °C, 9 °C	1.06	0.3703n.s.	1.04	0.401n.s.	1.02	0.423n.s.	1.06	0.3763n.s.
4 °C, 15 °C	1.25	0.2206n.s.	1.26	0.2125n.s.	1.35	0.1649n.s.	1.16	0.2852n.s.
4 °C, 25 °C	1.68	0.0546n.s.	1.48	0.109n.s.	2.24	0.0171*	1.43	0.1242n.s.
4 °C, 35 °C	3.90	0.0034**	3.10	0.006**	4.92	0.0012**	2.78	0.0082**
	Site 3 N		Site 3 S		Site 4 N		Site 4 S	
Overall ($F_{4,14}$, P)	10.63	<0.0001***	8.45	<0.0001***	8.48	<0.0001***	5.24	<0.0001***
Pairwise	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)
4 °C, 9 °C	1.45	0.1193n.s.	1.13	0.3146n.s.	1.10	0.3502n.s.	1.05	0.3969n.s.
4 °C, 15 °C	2.42	0.0143*	1.47	0.1087n.s.	1.69	0.0594n.s.	1.28	0.1909n.s.
4 °C, 25 °C	3.12	0.0048**	2.05	0.0238*	1.87	0.0478*	1.79	0.0462*
4 °C, 35 °C	3.56	0.0042**	3.89	0.0038**	4.61	0.0018**	3.69	0.0035**
RNA (PERMANOVA)								
	Site 2 N		Site 2 S		Site 4 N		Site 4 S	
Overall ($F_{4,14}$, P)	9.96	<0.0001***	5.10	<0.0001***	8.78	<0.0001***	5.87	<0.0001***
Pairwise	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)	$t_{1,4}$	P(MC)
4 °C, 9 °C	0.97	0.4722n.s.	1.03	0.4026n.s.	0.99	0.4497n.s.	0.95	0.4953n.s.
4 °C, 15 °C	1.21	0.2518n.s.	1.17	0.2794n.s.	1.47	0.1113n.s.	1.21	0.2484n.s.
4 °C, 25 °C	1.99	0.0289*	1.53	0.0898n.s.	1.94	0.033*	1.45	0.1203n.s.
4 °C, 35 °C	4.58	0.0013**	3.47	0.0039**	4.50	0.0016**	3.75	0.0029**
Comparison of community structures at the DNA and RNA level (Mantel test)								
	Site 2 N		Site 2 S		Site 4 N		Site 4 S	
	r	P	r	P	r	P	r	P
	0.992	<0.001 ***	0.973	<0.001 ***	0.974	<0.001 ***	0.924	<0.001 ***

Beta-diversity (differences in community structures based on Bray-Curtis dissimilarity) was analyzed in the subset for each soil using permutational multivariate analysis of variance (PERMANOVA). For pairwise comparisons, Monte Carlo simulations were used. Indices for F- and t-values indicate the numerator and residual degrees of freedom. Mantel test was used to compare community structures at the DNA and at the RNA level. n.s. not significant; *P < 0.05; **P < 0.01; ***P < 0.001. P (MC) = Monte Carlo P-value. Altitude of the sites: Site 1: 2460 m a.s.l., Site 2: 2780 m a.s.l., Site 3: 2960 m a.s.l., Site 4: 3070 m a.s.l. N = North, S = South.

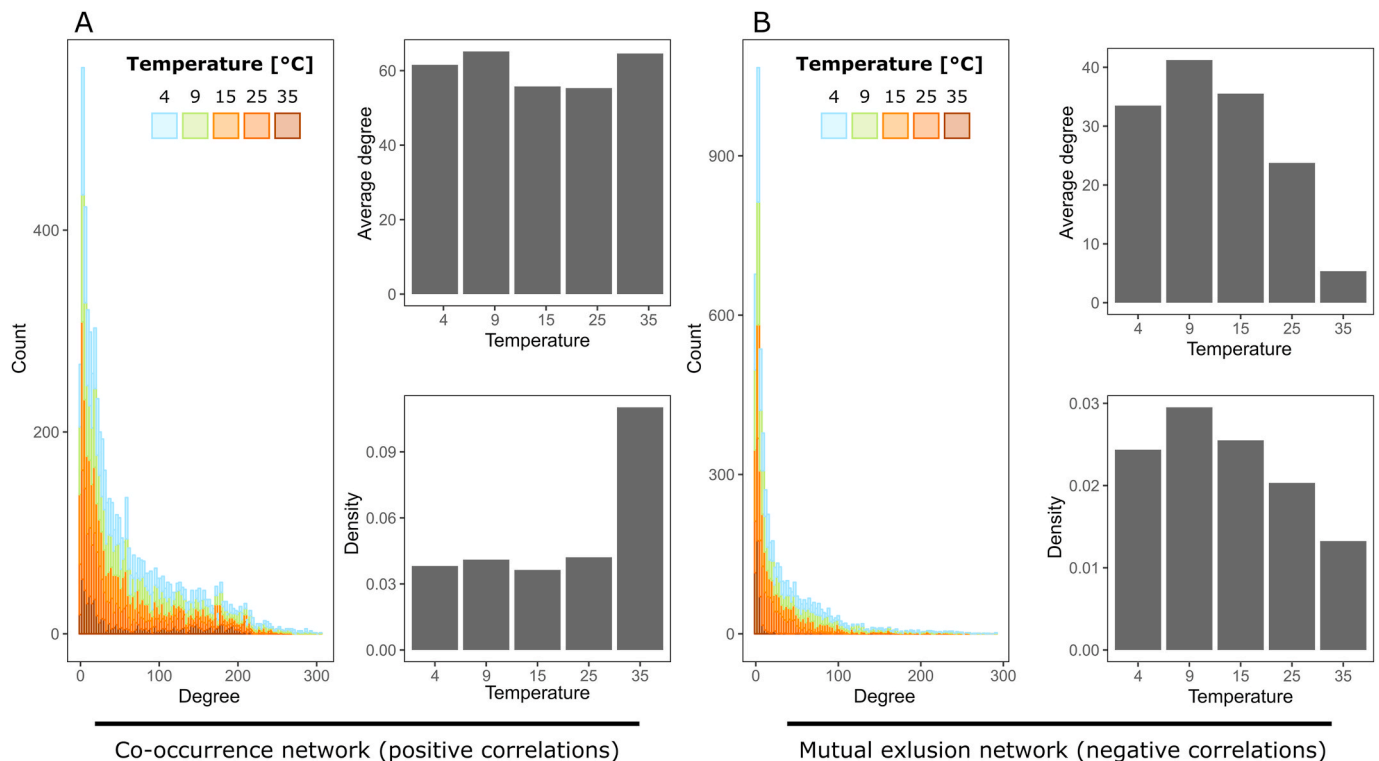


Fig. 3. Temperature effects on network properties. Network parameters of co-occurrence (A) and mutual exclusion networks (B). Relative abundances of OTUs were correlated using Spearman rank correlations at each temperature across all soils. For each type of network the degree distribution (left), the average degree across all nodes in the network (top right) and the network density (bottom right) are shown.

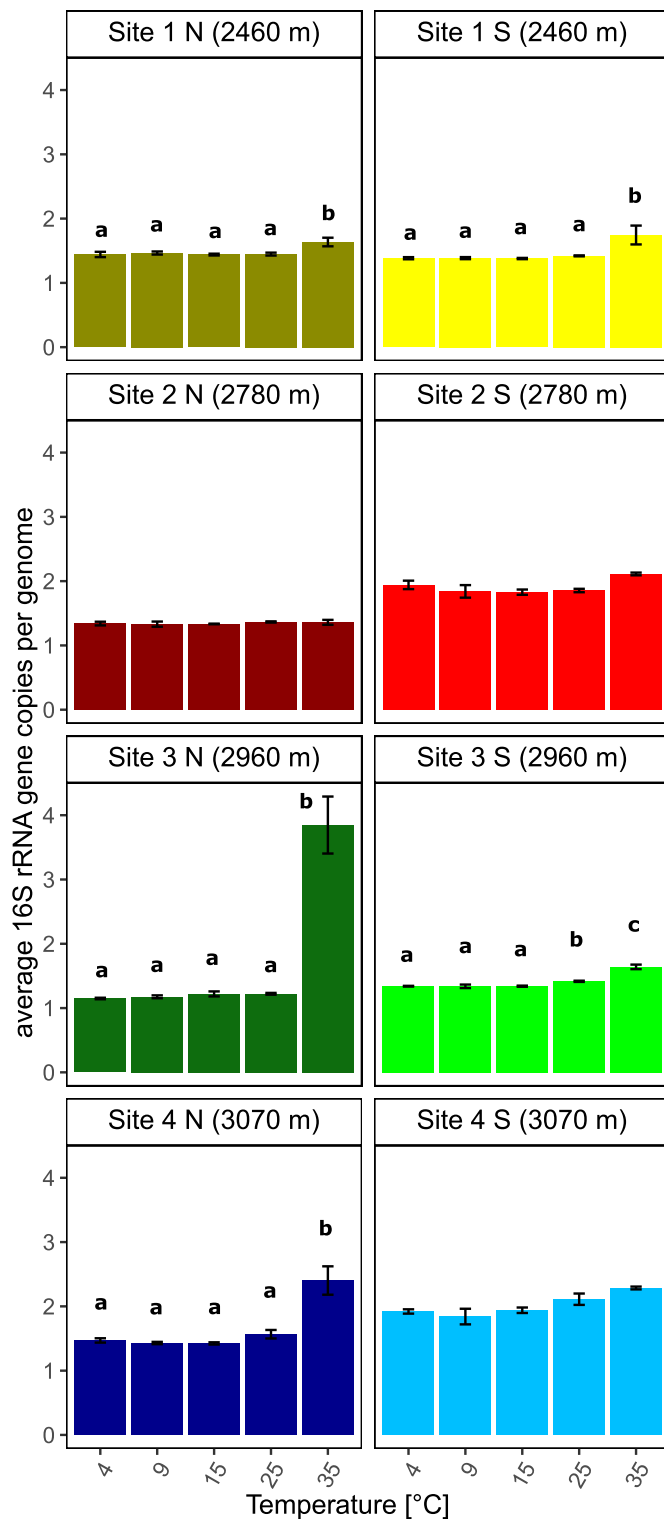


Fig. 4. Temperature effects on the trophic strategy. Average 16S rRNA gene copy number per bacterial genome (mean ± SD, n = 3) at different incubation temperatures as a proxy for the predominant trophic strategy (copiotrophs versus oligotrophs) in the community are shown. Different letters indicate a significant ($P < 0.05$) difference between temperatures as assessed by one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test in the subsets per soil.

the soils and that were not significant in the combined analysis. For example, at 35 °C, *Flaviatela* increased in site 4 N but decreased in site 2 S.

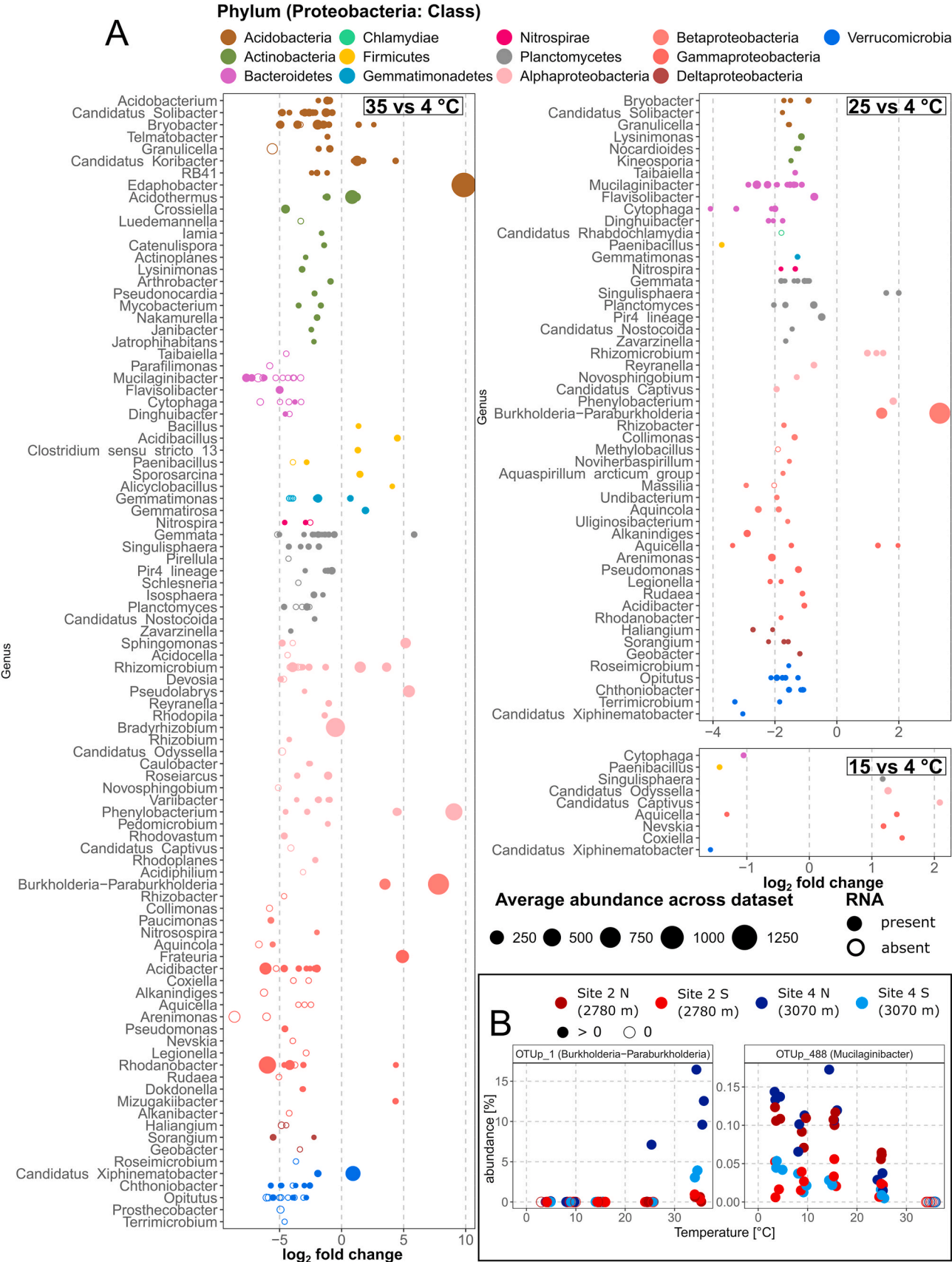
4. Discussion

4.1. Temperature adaptation of bacterial communities

Here, for the first time, we report on the temperature adaptation of bacterial growth in alpine soils from the alpine up to the nival zone. The range of T_{min} and T_{opt} in the different soils was between those for Antarctic soils (Rinnan et al., 2009) and those for temperate environments (Birgander et al., 2018; Nottingham et al., 2019). Correspondingly, MAST ranged between that from Antarctic and temperate soils. However, the increase in T_{min} per °C MAST (0.71 °C) is higher than in previous studies (Rinnan et al., 2009; Nottingham et al., 2019). The Q_{10} values found here were comparable to those reported by Rinnan et al. (2009) in Antarctic soils. In accordance with previous studies, T_{opt} was consistently 5–15 °C higher than the maximum recorded field soil temperature (Fig. 6A; Rinnan et al., 2009) and the temperature profile did not change when applying incubation temperatures significantly below the time 0 T_{opt} (Barcenas-Moreno et al., 2009; Birgander et al., 2013). Similarly, bacterial diversity and community structures remained constant with incubation temperatures up to T_{opt} (Fig. 6B). Conversely, temperatures in the range of T_{opt} (25 °C) and above (35 °C) caused a shift in the temperature dependency for bacterial growth towards higher temperatures, along with a strong decrease in diversity and a pronounced shift in community structures (Fig. 6C). Temperatures above T_{opt} exert high selective pressure on microorganisms (Barcenas-Moreno et al., 2009; Rinnan et al., 2009; van Gestel et al., 2013) and selection for taxa adapted to the whole range of *in-situ* temperatures can be expected (Wallenstein and Hall, 2012). Accordingly, temperature characteristics for microbial growth did not change seasonally (van Gestel et al., 2013; Birgander et al., 2018) nor in response to winter warming (Birgander et al., 2018). Conversely, permanent field warming by 5 °C led to an increase in T_{min} and Q_{10} (Rousk et al., 2012), similar to incubation temperatures above T_{opt} in the laboratory. The strong decrease in diversity at the highest incubation temperature underpins the selection process above T_{opt} where heat-sensitive taxa disappear, likely due to death or transition to dormancy. We can exclude that this pattern arose from a relative decrease in abundance alone, as total bacterial abundance remained constant across incubation temperatures. Patterns of diversity and community structures across incubation temperatures were comparable with previous studies in cold soils (Wu et al., 2015; Lulakova et al., 2019). In addition to previous studies, we found that a high proportion of OTUs was present at the RNA level at all incubation temperatures and communities at the RNA level showed highly similar changes compared to the DNA level. This confirms that our analysis was based on viable cells which has hampered the interpretability of previous microcosm studies due to the relative stability of DNA of lysed cells (Carini et al., 2016). Collectively, our results suggest that at short time scales the soil bacterial community is largely insensitive to temperature changes well within the range of *in-situ* temperatures and below T_{opt} . Conversely, the maximum *in-situ* temperature is a tipping point, above which temperature regimes lead to profound changes in both bacterial community structures and activity, which are likely associated with significant alterations in ecosystem functioning.

4.2. Temperature sensitivity depends on climatic legacy

We hypothesized that bacterial communities from colder soils react more sensitively to a given incubation temperature. This hypothesis was supported by a stronger decrease in diversity and a more pronounced change in community structures in colder soils, however only at 25 and 35 °C incubation temperature. At these temperatures in the range of or significantly above the community level T_{opt} (25 and 35 °C), direct



(caption on next page)

Fig. 5. Taxon level temperature effects. A) Differentially abundant OTUs compared to 4 °C at 35 °C (left), 25 °C (top right) and 15 °C (bottom right) at the DNA level. The soils where both DNA and RNA was analyzed (site 2 N and S, site 4 N and S) were included in the analysis and log₂-fold changes at each temperature were estimated controlling for the effect of site and aspect. Points represent individual OTUs. OTUs with a log₂-fold change significantly different from 0 (adj. $p < 0.05$) and classified at the genus level are shown. At 35 °C the 250 most abundant OTUs fulfilling these criteria are shown. Closed symbols represent OTUs that were found at the RNA level at the temperature that was compared to 4 °C. Open symbols represent OTUs that were found at the DNA level only. The size of the points indicates the average abundance of that OTU across all samples included in the analysis (raw count data normalized by the size factor). B) Changes in relative abundance across the temperature gradient (example of typical pattern) for strongly increasing (left) and decreasing OTUs (right). If the abundance was zero, the points are represented by open symbols to distinguish from very low abundance. More examples are given in Fig. S7.

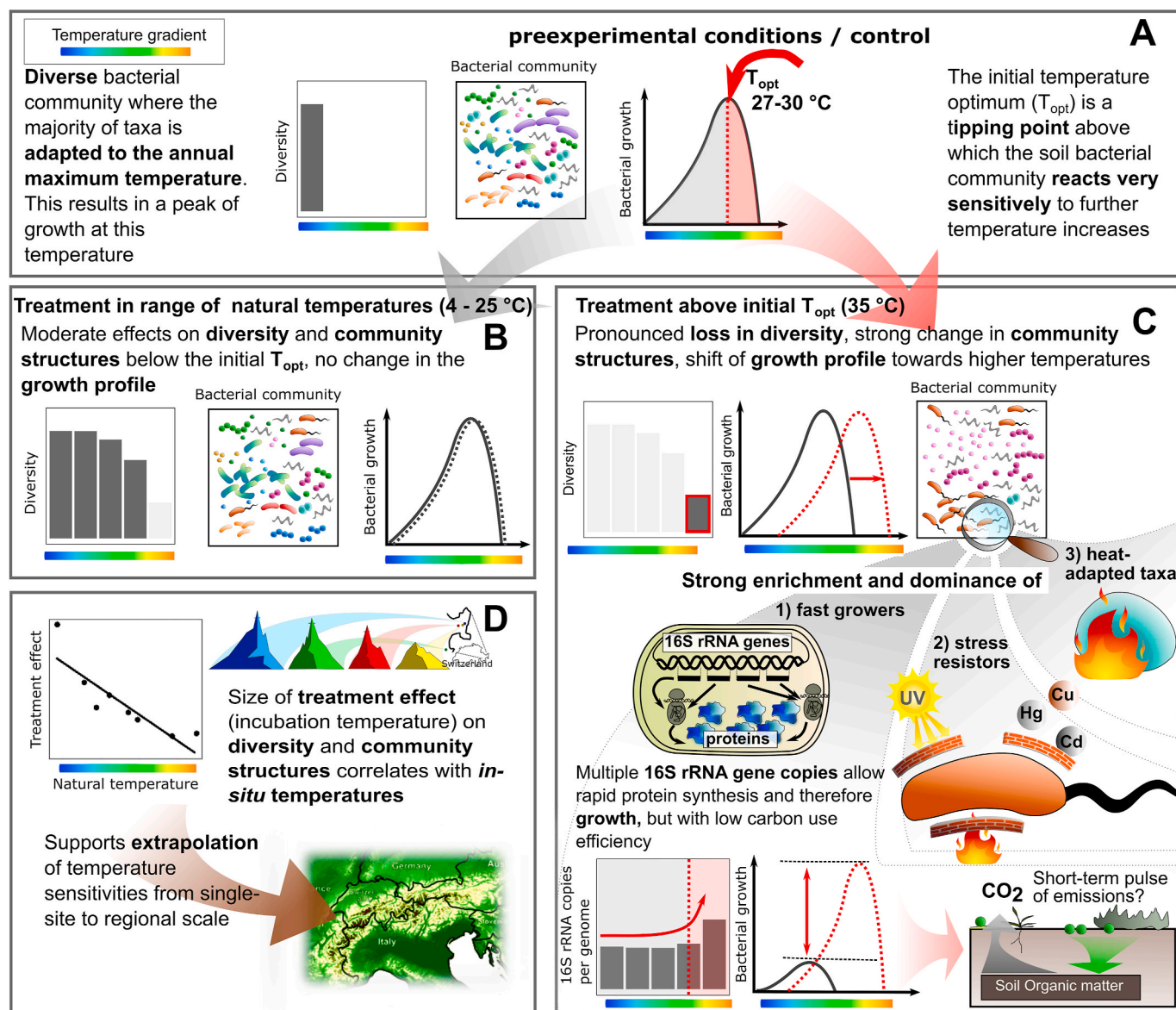


Fig. 6. Summary of the main findings of the study and their implications. A) Conditions before the experiment: the natural temperature optimum (T_{opt}) of bacterial growth represents a tipping point, determining whether bacterial communities are sensitive to the treatment. Incubation temperatures below T_{opt} lead to subtle responses (B) while temperatures above T_{opt} induce pronounced changes in bacterial community structures and the temperature dependency of growth (C). At the highest temperature fast growers, stress resistors and heat-adapted taxa become strongly enriched and dominate the community. An increase in fast growing taxa, which is associated with an overall increase in growth, might lead to a short-term increase in CO₂. In the long term, emissions are then expected to decline owing to depletion of substrate pools. D) The magnitude of the effect of the incubation temperature on bacterial communities correlated with *in-situ* temperatures. These results might help to up-scale temperature responses to a more regional level. Map modified after Gobiet et al. (2014).

temperature effects (i. e. temperatures exceeding the optimum of certain taxa slowing down growth and leading to cell death) explain this relationship. This is to say the magnitude by which T_{opt} for individual taxa is exceeded at a given temperature is greater on average in soils with lower *in-situ* temperatures. At temperatures <25 °C, we did not observe significant changes in diversity and community structures within the

incubation time of one month except in one soil. At longer time scales, substrate depletion effects are expected to affect the community, also at the temperatures that do not cause direct effects. It remains questionable how such effects would be related with the natural temperature regime, as both the size of the potential consumer community (16S rRNA gene abundance) and the amount of substrate (C content) were lower in soils

with lower *in-situ* temperatures before the incubation. Overall, the relationship between the magnitude of treatment response and the climatic legacy found here provides an important first insight how different alpine soils react to warming with respect to each other (Fig. 6D). Since mountain soils exhibit a very pronounced small-scale heterogeneity, our results might help to extrapolate findings from single site studies to a more regional scale.

4.3. High temperature decreases mutual exclusion

Environmental stimuli, such as alterations in the prevalent temperature regime, not only directly affect the fitness of different taxa in the community, but also relationships between taxa within the community such as symbiotic and competitive behavior. Such interactions influence ecosystem services provided by the community (Foster and Bell, 2012; Bell et al., 2013). Our network analysis revealed that both positive relationships (co-occurrence) and negative relationships (mutual exclusion) did not occur randomly but followed a power-law distribution. This is a typical feature of biological networks and suggests the presence of few highly connected hubs (Barabási and Albert, 1999). Interestingly, the average degree (i. e. the number of connections per node) decreased strongly at high temperatures for the mutual exclusion network, which suggests a decrease in competition. These findings are in accordance with the hypothesis that under harsh environmental conditions (in our case high temperature), organisms show less antagonistic behavior in order to ensure survival (Bertness and Callaway, 1994). In addition, reduced mutual exclusion at 35 °C may be explained by a higher fraction of dormant taxa in response to high temperature stress allowing for coexistence of taxa that would outcompete each other if both were active. Network analyses based on correlations of abundances should be interpreted with care, as one cannot distinguish between true biological interaction and co-occurrence due to shared habitat preferences (Röttgers and Faust, 2018). Hence, for instance increased network density in the co-occurrence network at the highest temperature may also be explained by a strong selection for taxa with similar temperature preference across all soils instead of an increased tendency to interact with other taxa. Further experimental evidence is needed to corroborate interactions among members of the bacterial community.

4.4. High temperature increases copiotrophic properties

At the highest incubation temperature (35 °C), we found an increase in 16S rRNA gene copy numbers per genome in most of the soils, which indicates a shift towards a more copiotrophic community (Fig. 6C). Compared to oligotrophs, copiotrophs possess a higher copy number of the 16S rRNA gene, which is an essential component of protein synthesis and thus any cellular activity. Multiple gene copies presumably enable copiotrophs to capitalize rapidly on sudden availability of nutrients (Klappenbach et al., 2000; Roller et al., 2016). Increased C and nutrient availability owing to death and lysis of cells in response to high temperature (Bérard et al., 2012; Mooshammer et al., 2017) might stimulate the growth of copiotrophs in our study. Similarly, Schostag et al. (2019) found an increase in average 16S rRNA copy number per genome (and thus copiotrophy) after maintaining permafrost soils at thawed conditions for approximately two weeks, which they ascribed to increased nutrient availability in response to thaw. Moreover, reduced competition (as suggested by our network analysis) upon death or inactivity of heat-sensitive taxa might favor the dominance of fast growers (Zhang and Zhang, 2015; Abreu et al., 2019). Besides the increase in 16S rRNA copies per genome, rapid bacterial growth at 35 °C is supported by a strong increase in ³H-leucine incorporation at all measurement temperatures. In the short term, a more copiotroph dominated community might contribute to elevated CO₂ emissions due to high activity and low carbon use efficiency (Roller and Schmidt, 2015). In the long term, the initial increase in copiotrophs is expected to lead to a faster depletion of the pool of organic matter in the soil and thus lower CO₂ emissions.

Accordingly, long-term warming studies repeatedly found that the warming induced increase in respiration declined over time (Rustad et al., 2001; Melillo et al., 2002; Hartley et al., 2007) which was attributed to substrate depletion (Kirschbaum, 2004; Hartley et al., 2008). Likewise, overall copiotrophy (rRNA gene copies per genome; DeAngelis et al., 2015) as well as bacterial growth rates (Rinnan et al., 2011; Rousk et al., 2012) decreased under long-term field warming. In addition, under *in-situ* conditions, initially increased respiration might be compensated by enhanced plant growth, which can be stimulated by elevated availability of inorganic nitrogen in response to warming (Melillo et al., 2002; Dawes et al., 2017).

4.5. Taxon-level shifts in the bacterial community

Community structures in the different soils shifted in parallel with incubation temperature indicating that the soils responded similarly to temperature. By identifying differentially abundant OTUs, we were moreover able to detect individual taxa that were significantly influenced by the incubation temperature across soils. Also at the taxon level, we found highly similar patterns at the DNA and RNA level and for all OTUs that increased with incubation temperature at the DNA level, presence at the RNA level was confirmed. These findings additionally corroborate that our analyses were based on viable cells. Total 16S rRNA gene copy numbers remained constant across all incubation temperatures. Therefore, we expect changes in absolute abundance to be similar to changes in relative abundance. The separate analysis of log₂ fold changes in the different soils (Fig. S10) revealed consistent direction of change across soils although with varying magnitude across both soils and replicates. This suggests that overall temperature favors the same taxa in different soils. Following disturbances involving partial eradication of local populations, stochastic recolonization plays an important role in community assembly (Fukami, 2015). In our study, where the temperature pressure is ongoing, likely, qualitatively, the temperature treatment determines the taxa becoming dominant in the community. However, similar to in post-disturbance community assembly, stochastic processes might determine quantitatively the success among surviving taxa to colonize newly available niches. Hence, stochasticity might explain varying changes in abundance with temperature across replicates and soil types. Similarly, Jurburg et al. (2017b) highlighted stochastic processes immediately upon heat disturbance.

Most differentially abundant OTUs were found at 35 °C and some of the genera favored at this temperature (*Burkholderia-Paraburkholderia*, *Phenylobacterium*, *Pseudolabrys*) have been found to respond positively to heat shock treatment previously (Jurburg et al., 2017a, 2017b). Overall, the enrichment of taxa at the highest treatment temperature may be promoted by *a priori* adaptation to this temperature, high growth potential as well as efficient mechanisms to withstand high temperature stress (Fig. 6C). *A priori* adaptation is supported by a shift in the optimum of bacterial growth towards the treatment temperature. Accordingly, mesophiles to moderate thermophiles such as *Alicyclobacillus* and *Acidibacillus* (both Firmicutes; Goto et al., 2007; Holanda et al., 2016) increased at 35 °C. Yet, several of the enriched genera (e.g. *Edaphobacter*, *Phenylobacterium* and *Burkholderia-Paraburkholderia*) have a temperature optimum around 30 °C according to the description of isolates (Coenye et al., 2001; Koch et al., 2008; Abraham et al., 2014). Transferring temperature relationships of isolates to environmental microorganisms can however be misleading, as the temperature range and optimum can vary significantly within the same species (Mongold et al., 1999; Coenye et al., 2001; Johnson et al., 2006). Stress tolerance as a mechanism of enrichment at high temperature is supported, as all genera comprising OTUs that increased strongly at 35 °C have been associated with harmful conditions such as heat shock as well as hydrocarbon and heavy metal contaminated soils (Frossard et al., 2017; Jurburg et al., 2017a, 2017b; Pires et al., 2017; Hemmat-Jou et al., 2018; Kou et al., 2018). Further, high temperature associated genera survived transport through the atmosphere (*Sphingomonas* and

Phenylobacterium (Šantl-Temkiv et al., 2013; Krumins et al., 2014)) and were able to thrive on rock surfaces (*Edaphobacter* (Esposito et al., 2015)) and to resist UV-radiation (*Sphingomonas* (Joux et al., 1999)). Such stressful conditions have in common that they cause misfolding of proteins, membrane disruption, oxidative stress and DNA damage (Nies, 1999; Santos et al., 2012; Fashola et al., 2016; Marcen et al., 2016). Owing to the similar impacts of different stressful cues, a universal defense mechanism is induced via heat-shock proteins upon the detection of damaged biomolecules rendering cross-resistance a common phenomenon although also specific stress responses exist (Inbar and Ron, 1993; Ramos et al., 2001; Ron, 2013). Increased stress tolerance can be achieved by alterations in the membrane structure to maintain integrity, enhanced protein stability through chaperones as well as repair or degradation and re-synthesis of damaged biomolecules (Ramos et al., 2001; Denich et al., 2003; Mykytczuk et al., 2007; Ron, 2013).

Among the genera enriched at high temperature, *Burkholderia-Paraburkholderia* represents a typical copiotroph. Therefore, we suggest that the pronounced increase in OTUs affiliated with *Burkholderia-Paraburkholderia* is linked to the overall increase in copiotrophy at 35 °C. This genus grows on simple sugars (Coenye, 2014) and was shown to increase in abundance upon addition of simple sugars (Jenkins et al., 2010; Goldfarb et al., 2011) and under conditions of elevated root exudation (Drigo et al., 2009) in environmental samples. *Burkholderia-Paraburkholderia* thrives in a large range of environments including polar and alpine soils (Lipson, 2007; Schutte et al., 2009; Ciccazzo et al., 2014). The ubiquity of *Burkholderia-Paraburkholderia* in various soil types is probably owed to the large variety of functions it performs such as N₂ fixation, degradation of a large range of organic compounds including cellulose and lignin as well as interaction with plants (both beneficial and pathogenic; Lim et al., 2003; Compant et al., 2008; Schutte et al., 2009; Stursová et al., 2012; Woo et al., 2014).

Among the taxa diminishing with increasing temperature, in line with previous studies, OTUs within the phylum Bacteroidetes decreased both under moderate (15, 25 °C) and strong warming (35 °C; Riah-Anglet et al., 2015; Oliverio et al., 2017). Bacteroidetes are ubiquitous in soil and have been associated with both oligotrophy and copiotrophy (Fierer et al., 2007; Männistö et al., 2016). Within this phylum, the genus *Mucilaginibacter* which comprised the most strongly decreasing OTUs is commonly found in cold environments (Männistö et al., 2010; Lee et al., 2011; Zheng et al., 2016) and the *Mucilaginibacter* species found in this study (*M. pineti*, *M. ximonensis* and *M. koreensis*) do not grow above 33 °C (Luo et al., 2009; Paiva et al., 2014; Park et al., 2014). Moreover, *Mucilaginibacter* is able to produce exopolysaccharides as cryoprotectants in frozen soils (Nikrad et al., 2016). Hence, adaptation to low temperatures might explain its sensitivity to high temperatures. The genus *Cytophaga* (Bacteroidetes), that also comprised OTUs which strongly decreased at 35 °C, is commonly found in Antarctic soils (Pearce et al., 2012) suggesting cold adaptation. *Mucilaginibacter* and *Cytophaga* play an important role in mineralizing organic matter, as they are able to degrade polysaccharides such as cellulose and chitin (Kirchman, 2002; Nissinen et al., 2012; Stursová et al., 2012). OTUs affiliated with Planctomycetes also consistently decreased with increasing temperature. Planctomycetes have been associated with an oligotrophic lifestyle previously (Lauro et al., 2009; Ward, 2010). For instance, the genus *Planctomycetes* was and indicator of soils low in C and N (Wojcik et al., 2018). Hence, their decrease might also contribute to the overall increase in copiotrophy at 35 °C.

5. Conclusion

Collectively, we showed that at short time scales only high temperatures (>25 °C) exert a significant effect on alpine soil microbial communities. Obviously, a continuous treatment at 35 °C for one month is not a realistic simulation of climate change. However, this approach allowed us elicit the range of temperatures across which bacterial communities remain stable or react sensitively. Applying our treatment

on a time scale similar to previous incubation studies (e. g. Barceñas-Moreno et al., 2009; Birgander et al., 2013; de Scally et al., 2016), we were able to link microbial activity and community structures and comparably embed our findings in the context of previous literature. A logical next step is to determine the role of duration and frequency with which a high temperature is applied to evaluate the sensitivity of the alpine soil microbiome to extreme heat events. At the community level, previous studies preliminarily indicate that microbial community structures react sensitively to extreme heat at scales ranging from less than one hour to a few days (Chaer et al., 2009; van der Voort et al., 2016). In addition, it will be crucial to assess the resilience of the soil microbiome to single or repeated pulses of heat in order to understand climate change impacts on the functioning of alpine ecosystems. Here, we provide an important baseline for such follow-up studies, as we determine the temperature threshold above which the soil bacterial community is strongly compromised by direct temperature effects. For the first time, we link temperature profiles of microbial activity with high resolution taxonomical shifts across multiple alpine soils showing that high temperature favors heat-adapted, fast growing and stress resistant taxa. Thus, we contribute to a more mechanistic understanding of climate change responses of the alpine soil microbiome.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2020.107873>.

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